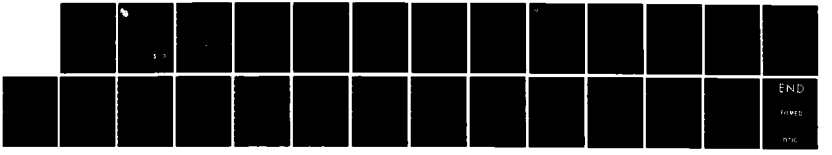
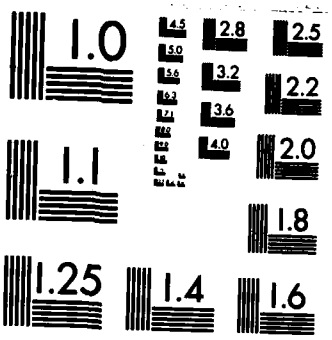
AD-A155 072 MUTAGENIC POTENTIAL OF GUANIDINE HYDROCHLORIDE (TP028) 1/1
(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN
FRANCISCO CA S K SANO ET AL. 02 MAR 85 LAIR-197

UNCLASSIFIED F/G 6/20 NL





INSTITUTE REPORT NO. 197

MUTAGENIC POTENTIAL OF GUANIDINE HYDROCHLORIDE (TPO28)

STEVEN K. SANO, BA, SP4
THOMAS P. KELLNER, BA, SP5
and
DON W. KORTE JR, PhD, MAJ MSC

TOXICOLOGY GROUP
DIVISION OF RESEARCH SUPPORT

MARCH 1985

Toxicology Series 80

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Mutagenic Potential of: Guanidine Hydrochloride (TP028) (Toxicology Series 80)-- Sano, Kellner and Korte

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

2 May 85
(Signature and date)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER LAIR Institite Report No. 197	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Mutagenic Potential of: Guanidine Hydrochloride (TP028)		5. TYPE OF REPORT & PERIOD COVERED Final 6 - 27 February 1984
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Steven K. Sano, BA, SP4 Thomas P. Kellner, BA, SP5 Don W. Korte, Jr., PhD, MAJ MS		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3E162720A835, WU 180
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701-5012		12. REPORT DATE 2 March 1985 13. NUMBER OF PAGES 25
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

THIS DOCUMENT HAS BEEN CLEARED FOR PUBLIC RELEASE AND SALE: ITS DISTRIBUTION IS UNLIMITED.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Mutagenicity, Toxicology, Ames Assay, Guanidine Hydrochloride (TP028)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of guanidine hydrochloride (TP028) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 ml/plate to 0.0016 mg/plate. Negative mutagenic responses were observed for the test compound.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ABSTRACT

The mutagenic potential of guanidine hydrochloride (TP028) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 mg/plate to 0.0016 mg/plate. Negative mutagenic responses were observed for the test compound.

Key Words: Mutagenicity, Toxicology, Ames Assay, Guanidine Hydrochloride (TP028)

PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Medical Bioengineering Research and
Development Laboratory
Fort Detrick, MD 21701-5012

WORK UNIT: 3E162720A835 Nitrocellulose-Nitroguanidine
Projects; WU 180; APC TL09

GLP STUDY NUMBER: 84007

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD

PRINCIPAL INVESTIGATOR: SP4 Steven K. Sano, BA

CO-PRINCIPAL INVESTIGATOR: SP5 Thomas P. Kellner, BA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, retired SOPs and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Guanidine Hydrochloride (TP028)

INCLUSIVE STUDY DATES: 6 - 27 February 1984

OBJECTIVE: The objective of this study was to determine the mutagenic potential of guanidine hydrochloride (LAIR code TP028).

ACKNOWLEDGMENTS

The authors wish to thank SP5 Lawrence Mullen, BS, and John Dacey for their assistance in performing the research.

Signatures of Principal Scientists Involved
in the Study

We, the undersigned, believe the study number 84007 described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply, with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

DON W. KORTE, JR., Ph.D. / DATE
MAJ, MSC
Study Director

STEVEN K. SANO, B.A. / DATE
SP4, USA
Principal Investigator

THOMAS P. KELLNER, B.A. / DATE
SP5, USA
Co-Principal Investigator

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

31 Jul 84

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 84007 the following inspections were made:

22 Feb 84

The report and raw data for this study were audited on 23 Apr 84.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 31 Mar 84 report to Management and the Study Director.

NELSON R. POWERS, Ph.D.
DAC
Chief, Quality Assurance Unit

TABLE OF CONTENTS

Mutagenic Potential of: Guanidine Hydrochloride (TP028)
-- Sano et al

The Ames Salmonella/Mammalian Microsome Mutagenicity Assay is a short-term screening assay that utilizes histidine auxotrophic mutant strains of Salmonella typhimurium to detect those compounds which are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the assay to increase sensitivity by simulating in vivo metabolic activation of the test compound. The Ames assay is an inexpensive yet highly predictive and reliable assay for detecting mutagenic activity and thus carcinogenic potential (1).

Objective of the Study

The objective of this study was to determine the mutagenic potential of guanidine hydrochloride (LAIR code TP028).

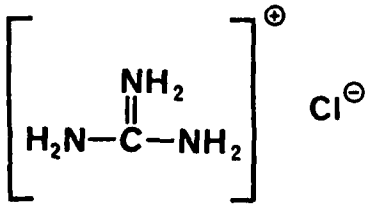
METHODS

Test Compound

Chemical name: Guanidine Hydrochloride

Chemical Abstract Service Registry No.: 50-01-1

Structural formula:



Empirical formula: $CH_5N_3 \cdot HCl$

Storage: One kilogram of guanidine hydrochloride (Lot Number 103-F-5623) was obtained from the Sigma Chemical Co. (St. Louis, MO) in December 1983 and assigned the LAIR Code number TP028. The test compound was stored at room temperature (21°C).

Chemical Properties/Analysis: Data characterizing the chemical composition was obtained from Sigma Chemical Co. (St Louis, MO) and the stability analysis of the test material was performed by the Toxicology Services Group, LAIR (Presidio of San Francisco, CA), (Appendix A).

Test Solvent

Positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 100F-0269) obtained from Sigma Chemical Co. (St. Louis, MO).

Test compound was dissolved in sterile, deionized water obtained from a Polymetrics Model 200-3 Water Purifer (Sunnyvale, CA).

Chemical Preparation

On the day before dosing, 300 mg of the test compound was measured into a sterile vial and again stored at room temperature. On the day of dosing, the 300 mg sample was dissolved in a 6 ml volume of autoclaved water from a polymetrics water filter system to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates. The dosing procedure was completed within 20 minutes of dissolving the test compound.

Test Strains

Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538, obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality controls were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (2).

Test Form t

Guanidine hydrochloride was evaluated for mutagenic potential according to the methods of Ames et al (3). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (2).

Toxicity Tests

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of guanidine hydrochloride ranging from 1.6×10^{-3} mg/plate to 5 mg/plate, and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin were placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since none of the plates showed decreased macrocolony formation (below the level of the spontaneous reversion plates) or an observable reduction in the density of the background lawn, a maximum "limit" dose of 5 mg per plate was used in the mutagenicity assay.

Mutagenicity Assay

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5 both with and without 0.5 ml of the S-9 microsome fraction. The S-9 was purchased from Litton Bionetics (Kensington, MD). The optimal titer of this S-9, as determined by Litton Bionetics, was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and in all reagents came from a polymetric system. Plates were incubated, upside down in the dark, at 37°C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). No vehicle control was required as the test compound was diluted in water. The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound assay. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound assay plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Ames et al (3). Concurrent sterility and strain verification controls were run. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The Salmonella strains were verified by a standard battery of tests.

The following tests were run to determine if:

- Lipopolysaccharide layer (LP) alteration causes growth inhibition in the presence of crystal violet.

- An ampicillin-resistant R factor has allowed growth in strains TA 98 and TA 100 in the presence of ampicillin impregnated disks.

- Absence of excision repair mechanism has inhibited growth in the presence of ultraviolet light.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. These compounds, benzo [a] pyrene, 2-aminofluorene, 2-aminoanthracene and N' methyl-n-'nitro-n-nitrosoguanidine, were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Ames et al (3), a compound is considered mutagenic if the following criteria are met:

- The test compound causes revertant colony counts greater than or equal to twice the spontaneous revertant rate.

- The test compound produces a correlated dose response relationship.

RESULTS

On 15 February 1984, the toxicity level determination was performed on guanidine hydrochloride (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 2). No toxicity was observed after exposure of the tester strain (TA100) to the highest dose used (5 mg/plate).

Normal results were obtained for all sterility, strain verification, and negative controls during the Ames Assay performed on 21-23 February 1984 (Tables 3-4). No revertant counts were obtained that exceeded double the spontaneous reversion rate following exposure of the bacterial strains to the test compound (Table 5).

TABLE 1

TOXICITY LEVEL DETERMINATION

Substance assayed: GUANIDINE HYDROCHLORIDE Substance dissolved in: H_2O

Study Number: 84007 Date: 17 February 1984 Performed by: MULLEN, KELLNER

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
TPO28 5 mg/pl	107	109	94	103	NL
1 mg/pl	124	114	104	114	NL
0.2 mg/pl	124	114	104	114	NL
0.04 mg/pl	98	120	100	106	NL
0.008 mg/pl	106	119	117	114	NL
0.0016 mg/pl	97	108	111	105	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 2

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	NG (20 mm)	NG	+
Wild Type	G	NT	G	NT	NT	+

STERILITY CONTROL FOR TOXICITY LEVEL DETERMINATION

His-Bio Mix Initial: NG End: NG MGA Plate: NG

Top Agar Initial: NG End: NG

Diluent: NG Nutrient Broth: NG

Test Compound (a) NG (b)_____ (c)_____ (d)_____ (e)_____

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9

(1) + = expected response - = unexpected response

Study Number: 84007 Date: 16 Feb 1984 By: KELLNER, SANO

TABLE 3

STRAIN VERIFICATION CONTROL FOR ASSAY

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	NG (20 mm)	NG	+
100	NG	G	NG	NG (25 mm)	NG	+
1535	NG	NT	NG	NG (26 mm)	NG	+
1537	NG	NG	NG	NG (26 mm)	NG	+
1538	NG	NT	NG	NG (25 mm)	NG	+
WT	NT	NT	G	NT	NT	+

STERILITY CONTROL FOR ASSAY

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: w S-9 NG / w/o S-9 NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound (a) NG (b)_____ (c)_____ (d)_____ (e)_____ (f)_____

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 84007 By: SANO, KELLNER

Date: 23 February 1984

(1) + = expected response
- = unexpected response

TABLE 4

POSITIVE AND NEGATIVE CONTROL TEST

(Revertants/Plate)
Mean

Compd.*	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
AF	2 ug/plate	yes	(467,416,230) 371	(292,268,241) 267			(348,308,326) 327
BP	2 ug/plate	yes	(104,114,100) 106	(598,650,640) 629		(89, 67, 70) 75	(124,100,155) 126
AA	2 ug/plate	yes	(1397,1489,1331) 1406	(1643,1547,1621) 1604		(188,173,100) 154	(955,1187,1122) 1088
MNNG	2 ug/plate	no		(1607,1519,1520) 1549			
	20 ug/plate	no			(1556,1576,1578) 1570		
Spontaneous Reversion Rate/ Negative Control							
	before assay	yes	(32, 30, 30)	(139,114,140)	(18, 19, 19)	(12, 8, 5)	(12, 23, 19)
	after assay		(26, 26, 17) 27	(126,133,126) 130	(25, 16, 18) 19	(5, 4, 6) 7	(17, 14, 15) 17
	before assay	no	(20, 15, 23)	(126,112,116)	(36, 28, 36)	(6, 6, 8)	(11, 15, 12)
	after assay		(25, 10, 14) 18	(142, 84,108) 115	(25, 18, 16) 27	(2, 4, 8) 6	(14, 13, 15) 13

Study Number: 84007

Date: 24 Feb 1984 By: KELLNER, SANO

*Compounds: AF = 2-aminofluorene, BP = Benzo(a)pyrene, AA = 2-aminoanthracene, MNNG = N-methyl-n'-nitro-n-nitrosoguanidine

†$$$ Indicates colony counts exceeded 1000

TABLE 5

GUANIDINE HYDROCHLORIDE ASSAY

(Revertants/Plate)
Mean

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
TP028	5 mg/pl	yes	(28, 40, 29) 32	(118,136,131) 128	(19, 20, 25) 21	(6, 6, 4) 5	(18, 11, 17) 15
		no	(26, 21, 18) 22	(122,139,120) 127	(30, 28, 31) 30	(5, 8, 6) 6	(10, 7, 10) 9
	1 mg/pl	yes	(36, 29, 24) 30	(133,125,173) 144	(22, 21, 27) 23	(8, 8, 5) 7	(19, 15, 26) 20
		no	(17, 23, 14) 18	(144,111,105) 120	(34, 31, 26) 30	(9, 4, 6) 6	(14, 15, 14) 14
	0.2 mg/pl	yes	(40, 27, 26) 31	(137,150,134) 140	(20, 17, 14) 17	(8, 5, 5) 5	(10, 19, 23) 17
		no	(21, 15, 15) 17	(19, 25,141) 62	(6, 18,CON) 12	(4, 7, 5) 5	(10, 9, 9) 9
	0.04 mg/pl	yes	(35, 33, 17) 28	(112,132,114) 119	(19, 14, 16) 16	(6, 6, 8) 7	(13, 12, 16) 14
		no	(18, 25, 15) 19	(156,112,140) 136	(46, 52, 46) 48	(6, 4, 9) 6	(11, 11, 11) 11

Study No.: 84007 Date: 24Feb 1984 Performed by: SANO, KELLNER

TABLE 5 (concluded)

GUANIDINE HYDROCHLORIDE ASSAY

Compd	Amount of Compd. Added	S-9 Added	(Revertants/Plate) Mean Strain No. 98	100	1535	1537	1538
TP028	0.008 mg/pl	yes	(29, 28, 45) 34	(124,106,126) 119	(22, 16, 13) 17	(8, 3, 11) 7	(21, 26, 20) 22
		no	(16, 19, 26) 20	(127,108,102) 112	(34, 27, 36) 32	(5, 4, 3) 4	(11, 15, 13) 13
	0.0016 mg/pl	yes	(28, 24, 25) 26	(126,110,127) 121	(17, 19, 14) 17	(5, 10, 9) 8	(23, 9, 32) 21
		no	(18, 17, 8) 14	(140,123,138) 134	(41, 45, 31) 39	(3, 8, 6) 6	(22, 12, 12) 15

Study No.: 84007 Date: 24 Feb 1984 Performed by: SANO, KELLNER

DISCUSSION

Certain test criteria must be satisfied before an Ames assay can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, LP layer alterations, and DNA excision repair deficiencies. Second, the Salmonella strains must be responsive to the mutagenic process by exposing the strains to known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on macrocolony and microcolony formation. If these tests are performed and expected data are obtained, then the results of Ames test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, guanidine hydrochloride was evaluated by the Ames assay. In no instance did the test compound elicit a doubling of the spontaneous reversion rate or a correlated dose response relationship. Thus, the results of this study indicate guanidine hydrochloride is not mutagenic when evaluated in the Ames assay.

CONCLUSION

Guanidine hydrochloride is not mutagenic by the Ames assay at the dose levels tested.

RECOMMENDATION

Guanidine hydrochloride should undergo further toxicity tesing in accordance with the Toxic Substances Control Act.

REFERENCES

1. McCann JE, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 1975;72:5135-5139.

2. Ames Salmonella/Mammalian Microsome Mutagenicity Assay. LAIR Standard Operating Procedure OP-STX-1, Letterman Army Institute of Research, Presidio of San Francisco, California, 15 November 1983.

3. Ames, BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with Salmonella/Mammalian microsome mutagenicity test. Mutation Res 1975;31:347-364.

4. Vogel HJ, Bonner DM. Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem 1956;218:97-106.

APPENDIX

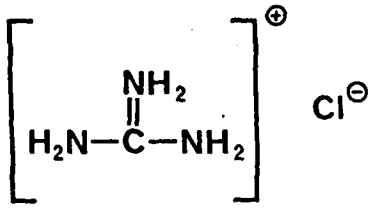
CHEMICAL DATA

Chemical Name: Guanidine Hydrochloride

Alternate Chemical Name: Aminomethanamidine hydrochloride,
Carbamamidine hydrochloride,
Carbamidine hydrochloride,
Aminoformamidine hydrochloride,
Iminourea hydrochloride

Chemical Abstracts Service Registry No.: 50-01-1

Chemical structure:



Molecular formula: CH_6ClN_3

Molecular weight: 95.5

Physical state: White powder

Melting point: 182-184°C (184-185°C*)

Analytical data/purity: Water content 0.1% by Karl Fischer analysis.*
The material is at least 98% pure and chromatographs as one spot by thin layer chromatography.†
Elemental analysis. Calculated for CH_6ClN_3
Cl, 37.1. Found: Cl, 36.6.† An IR spectrum was obtained upon receipt of the compound.
IR(KBr): 3400, 2750, 1650, 1535, 1050 (broad) cm^{-1}.
A comparison of this spectrum to the Sadtler standard spectrum confirmed the identity of the material.‡

Source: Sigma Chemical Co.
St. Louis, MO

Lot number: 103F-5623

---

*Zygmunt R., Analytical data sheet for guanidine hydrochloride, lot number 103F-5623. Sigma Chemical Co., St. Louis. 16 Feb 84.

†Sigma Chemical Company, St. Louis, MO. Becky Goodloe, PhD, personal communication, 5 March 1985.

‡Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #8676.

Stability in vehicle: A preliminary study was conducted to determine the stability of guanidine hydrochloride in the vehicle, sterile water for injection. A solution of guanidine hydrochloride (18.825 ug/ml water) was assayed after preparation and 4 hours later by using the Voges-Proskauer Method (Micklus MJ, Stein IM. The colorimetric determination of mono-and disubstituted guanidines. Anal Biochem 1973;54:545-553). This method is specific for unsubstituted and monosubstituted guanidines and yields a colored derivative which is monitored spectrophotometrically. Three samples were analyzed for each time point and the results were as follows:

Absorbance Value (1st Assay)	Absorbance Value (2nd Assay)
2.190	2.053
2.165	2.190
2.160	2.191
$\bar{x}$ = 2.172	$\bar{x}$ = 2.145

The values for the two assays were within 1.5 percent of each other which is within the error for repeated sampling using this test. This indicates that the compound is stable in aqueous solution for at least 4 hours.

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
and Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, Frederick MD 21701

Defense Technical Information Center
ATTN: DTIC-DDA (2 copies)
Cameron Station
Alexandria VA 22314

Director of Defense Research and Engineering
ATTN: Assistant Director, Environmental
and Life Sciences
Washington DC 20301

The Surgeon General
ATTN: DASG-TLO
Washington DC 20314

HQ DA (DASG-ZXA)
WASH DC 20310

Commandant
Academy of Health Sciences
ATTN: HSHA-CDM
Fort Sam Houston TX 78234

Assistant Dean
Institute and Research Support
Uniformed Services University
of Health Sciences
6917 Arlington Road
Bethesda MD 20014

Commander
US Army Environmental Hygiene Agency
Aberdeen Proving Ground MD 21070

US Army Research Office
ATTN: Chemical and Biological Sciences
Division
P.O. Box 1221
Research Triangle Park NC 27709

Biological Sciences Division
Office of Naval Research
Arlington VA 22217

Director of Life Sciences
USAF Office of Scientific Research (AFSC)
Bolling AFB
Washington DC 20332

Director
Walter Reed Army Institute of Research
Washington DC 20307

Commander
US Army Medical Research Institute
of Infectious Diseases
Fort Detrick, Frederick MD 21701

Commander
US Army Research Institute
of Environmental Medicine
Natick MA 01760

Commander
US Army Institute of Surgical Research
Brooke Army Medical Center
Fort Sam Houston TX 78234

Commander
US Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick, Frederick MD 21701

Commander
US Army Aeromedical Research Laboratory
Fort Rucker AL 36362

Commander
US Army Research Institute
of Chemical Defense
Aberdeen Proving Ground
Edgewood Arsenal MD 21010

Commander
USAF School of Aerospace Medicine
Aerospace Medical Division
Brooks Air Force Base TX 78235

END

FILMED

7-85

DTIC